Grafting of Technical Lignins Through Regioselective Triazole Formation on β-O-4 Linkages

Isabella Panovic,[a] James R. D. Montgomery,[a] Christopher S. Lancefield,[a] Dhivya Puri,[b] Tomas Lebl,[a] Nicholas J. Westwood[a]*

[a] School of Chemistry and Biomedical Sciences Research Complex, University of St Andrews and EaStCHEM, North Haugh, St Andrews, Fife KY16 9ST (UK). E-mail: njw3@st-andrews.ac.uk. [b] Fiberight Ltd., 32 Monk Street, Abergavenny, Gwent, NP7 5NW (UK).

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ABSTRACT A new method has been developed to enable the modification of the organosolv technical lignin. Using a walnut shell butanol alkoxasolv lignin as a source of high β-O-4 content material, the β-O-4 γ-position has been selectively modified via tosylation, **a**zidation and copper-catalyzed azide-alkyne triazole formation . In addition, extensive model studies were used to aid the detailed characterization of the modified lignin structure. The copper catalyzed click reaction was used to attach modified PEG chains and the resulting lignin-based co-polymer displayed improved thermal stability. This protocol was also used to incorporate a novel BODIPY-type

fluorophore, generating a fluorescent lignin. Copper catalytic loadings were effective as low as 0.3 weight% and were found to catalyze the cycloaddition efficiently. This efficient and generic approach to preparing lignin-derived polymers is relevant to the core societal challenge of improving biorefinery efficiency.

Introduction

Lignin, a heterogeneous biopolymer, is one of the main components of lignocellulosic biomass. Lignin is formed from the enzymatic polymerization of the phenylpropanoid units *p*-coumaryl, coniferyl and sinapyl alcohols,[1] making it a potentially valuable source of aromatic compounds. The combinatorial nature of lignin biosynthesis leads to the formation of a complex and heterogeneous biopolymer which contains a number of conserved linkage types, with the most abundant being the β-O-4 linkage, along with the β-β and β-5 units. Current interest in lignin valorization is very diverse but often involves its use in sustainable material applications or its depolymerization to useful aromatic chemicals.[2,3]

The application potential of the lignin fraction in biomass is often determined by which pretreatment is employed to liberate it from the cellulose and hemicellulose fractions. Industrial pretreatments, such as the Kraft or Lignosulfonate processes, yield a heavily modified lignin which is often degraded and retains few β-aryl ether linkages.[4] The industrial use of these lignins often involves returning their stored energy content back into the extraction process, often through burning. In contrast to these harsh extraction conditions, the use of organosolv extractions involving the heating of lignocellulosic biomass with an organic solvent, such as 1,4-dioxane,[5]

ethanol[6,7] or butanol[8], in dilute acid can often give more native lignin structures that include high yields of a β-O-4-rich lignin.

The incorporation of lignin into sustainable functional materials usually requires prior chemical modification to improve crucial properties, such as mechanical and thermal stability.[9,10] Commonly these chemical modifications involve derivatizing and 'grafting' to or from lignin's phenolic end-groups.[11,12] An example of this involves phenol propargylation of lignin which has been used as a substrate for thermal[13] and copper-catalyzed azide-alkyne cycloadditions (CuAAC).[14–16] Compared to the thermal 1,3-Huisgen cycloaddition approach which produces both the 1,4- and 1,5-regioisomers, often in an inseparable mixture, the CuAAC approach has the advantage of regioselectively forming only the 1,4-triazole regioisomer. In addition, the CuAAC chemistry is very flexible and we envisaged it could be used to derivatize lignin along its whole backbone (rather than just at the phenolic end groups). Possible applications include the use of fluorescent lignin in imaging/sensing applications;[17,18] the synthesis of lignin-antibiotic conjugates;[19] and use as a delivery mechanism in biological applications.[20]

In this work we modified the γ-hydroxyl of the β-O-4 unit in a butanol-extracted organosolv lignin, which we have termed butanosolv lignin here. Initially we introduced a tosyl group at this position (previously attempted in the literature[21–23]) enabling further chemical modification. Following tosylation of the biopolymer, substitution using azide enabled copper-catalyzed formation of a triazole-bridged connection from lignin to a desired substrate. The green modification of lignin in this way allowed us to tune its material properties and to introduce new properties which might increase its industrial value.

Experimental

Materials

The lignocellulosic feedstocks used throughout were obtained from the following suppliers: Fiberight Ltd. provided the Fiberight waste composite material, Douglas fir sawdust was obtained from Hot Smoked (Useful Stuff Ltd), UK and the ground walnut shells were a kind donation from A. & E. Connock, UK. All materials were used as received.

Methods

Butanosolv lignins were extracted following a literature protocol.[8]

For lignin tosylations, a solution of lignin (1 wt eq.) in pyridine (10 mL/g) with toluenesulfonyl chloride (3 wt eq.) was stirred at rt for 6 h, then added dropwise to 0.1 M HCl (10 v/v). The precipitated tosylated lignin was collected by filtration and dried in a vacuum oven at 50 ^{o}C for 16 h. The dried solid was dissolved in the minimum amount of acetone, then added dropwise into ethanol (10 v/v).

For the azidation of lignin, a solution of tosylated lignin (1 wt eq.) was stirred in DMF (10 mL/g) with NaN_3 (2 wt eq.) at 50 ^{o}C for 24 h, then cooled to rt. The reaction mixture was added dropwise to H_2O (10 v/v) and azidated lignin was collected either by filtration or by centrifugation (6000 rpm, 5 min) and dried in a vacuum oven at 50 ^{o}C for 16 h. Caution was taken when using sodium azide and organic azide due to possible explosion risks.

For the CuAAC reactions on lignin, a solution of azidated lignin (1 wt eq.), alkyne (0.5 wt eq.), $CuSO_4.5H_2O$ (0.03 wt eq.) and ascorbic acid (0.5 wt eq.) was stirred in DMF / H_2O (5:1, 10 mL/g) at rt for 16 h, then added dropwise to acidified H_2O (10 v/v). Precipitated lignin was collected by

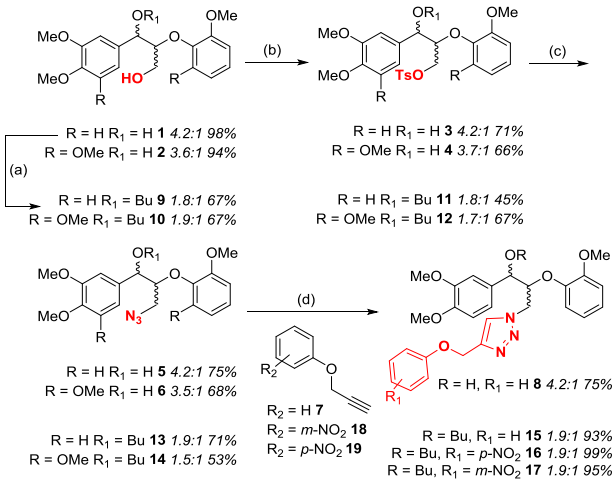
centrifugation (6000 rpm, 5 min), the eluent was decanted off, then the solid was dried in a vacuum oven at 50 ºC for 16h. The solid precipitate was then dissolved in acetone and precipitated into petroleum ether (10 mL/g), filtered off and dried in a vacuum oven at 50 ºC for 16 h.

See the Supporting Information for the synthesis and analytical characterization of all model compounds. 2D HSQC NMR spectra were acquired using a standard Bruker pulse sequence 'hsqcetgpsp.2' (phase-sensitive gradient-edited- 2D HSQC using adiabatic pulses for inversion and refocusing) was used. For a more detailed description of the 2D HSQC NMR protocol please see the General Consideration section of the ESI.

Results and Discussion

The chemical modification of the biopolymer lignin is complicated by difficulties in characterizing changes in its structure. Lignin's heterogeneous nature means that even the use of high-level characterization techniques such as 2D HSQC NMR can be less informative than would be ideal. It was therefore decided to begin this study by exploring the likely reactivity of lignin using dimeric β-O-4 model compounds **1-8** (**Schemes 1** and **S1**). The use of alcoholic solvents during acid-catalyzed organosolv pretreatment of biomass leads to incorporation of the alcohol at the benzylic position of the β-O-4 unit. For example, the incorporation of ethanol has been observed during ethanosolv pretreatment of Miscanthus.[6] In addition, we have recently reported that when high percentages of butanol are used in the liberation of lignin it is possible to achieve close to 100% incorporation of butanol at the benzylic position of the β-O-4 unit (to give a butanosolv lignin).[8] As it was unclear at the start of this work which lignin (a native β-O-4 containing or an α-

substituted alkoxasolv lignin) would prove optimal, it was also decided to assess our protocol using β-O-4 models **9-17** that contained an ether group at the benzylic position.



Scheme 1: Synthesis of β-O-4 models **1-17** using conditions; (a) ^{n}BuOH, cat. HCl, reflux, 15 mins.; (b) p-TsCl, Et_3N, DCM, rt, 6 h; (c) NaN_3, DMF, 50 ^{o}C, 24 h, (d) alkyne **7**/**18**/**19**, Cu(II)SO_4.5H_2O, sodium ascorbate, ^{t}BuOH/H_2O (3:1), rt, 4 h. Models **1**, **2**, **9** and **10** were synthesized according to previous reports.[8,24] Ratios shown in italics represent the diastereomeric ratio of the major and minor diastereomers, small changes in values occurred on purification by column chromatography. See **Scheme S1** for a more detailed representation.

Model Studies on selective β-O-4 functionalization

Two non-phenolic β-O-4 models **1** and **2** were used to model two homodimeric β-O-4 scenarios (G-G in model **1** and S-S in model **2**, **Schemes 1 and S1**). Both models were prepared as mixtures of diastereomers (*major:minor* ratio 4.2:1 for **1** and 3.6:1 for **2**[24]) using literature procedures.[24,25] As expected,[23,26] selective tosylation of the γ-primary alcohol in **1** and **2** was achieved to give **3** and **4** respectively. Whilst no direct evidence was available to assign the relative stereochemistry of the two diastereomers of **3** and **4**, it was assumed that this reaction occurred with no change at either

of the stereocentres based on the likely mechanism and the obtained diastereomeric ratios for the products (*major:minor* isomer ratio 4.2:1 for **3** (*c.f.* 4.2:1 for **1**) and 3.7:1 for **4** (*c.f.* 3.6:1 for **2**)). Subsequent reaction of **3** and **4** with sodium azide led to the formation of novel compounds **5** and **6** respectively (*major:minor* isomer ratio 4.3:1 for **5** and 3.5:1 for **6**). With **5** in hand, a copper-catalyzed 1,3-dipolar cycloaddition reaction (CuAAC)[15] was attempted using alkyne **7** to give 1,4-triazole **8** as a mixture of diastereomers (*major:minor* ratio 4.3:1) in 75% yield. The successful conversion of **1** to **8** suggested that our protocol could be applicable for the modification of a lignin biopolymer.

Models **9** and **10** were prepared by reaction of models **1** and **2** with butanol and catalytic hydrochloric acid.[8] In models **9** and **10**, a change in diastereomeric ratio (*major:minor* isomer ratio 1.8:1 for **9** (*c.f.* 4.2:1 for **1**) and 1.9:1 for **10** (*c.f.* 3.6:1 for **2**)) was observed. The tosylation and azidation steps proceeded without issue to yield models **13** and **14**. The subsequent CuAAC reaction of **13** with alkyne **7** to form **15** was also successful, suggesting that our protocol was also applicable for use with biorenewable alcohol-extracted lignins.

Incorporation of an azide functional group in lignin

The next step was to apply the protocol to lignin itself. After an extensive preliminary study (**Figures S1-S4** & **Tables S1-S3**), a **B**utanosolv **W**alnut shell **L**ignin (BWL) was selected as; (i) walnut shells are an abundant agricultural waste product; (ii) lignin solubility in organic solvents (THF, DCM, acetone) was excellent; (iii) high levels of the (modified) β-O-4 linkage were retained and (iv) this approach removed any concerns about 1°/2° alcohol selectivity when using lignin (although none were observed in model studies with **1** and **2**).[8] BWL was then treated under

modified tosylation reaction conditions compared to those described above for model compounds **1**, **2**, **9** and **10** (see below for more discussion). The success of these reactions on BWL was monitored using 2D HSQC NMR, GPC and FT-IR analysis. Changes in the 2D HSQC NMR chemical shift of the signals corresponding to the γ-CH_2 protons in the β-O-4 linkage were particularly diagnostic as highlighted in **Figures 1A** and **1B** (and **Figures S5** and **S6**). The changes in the NMR spectra observed following lignin tosylation also corresponded to previous observations in the literature (**Table S4**, **Figures 1A-D**) and were found to be highly reproducible (**Figure S7**).[21,23] In addition, significant changes were seen in the FT-IR spectrum, especially after the BWL azide substitution reaction. An indicative azide stretch at 2100 cm^{-1} was observed for the azidated BWL (**Figures 1E** and **S8**).

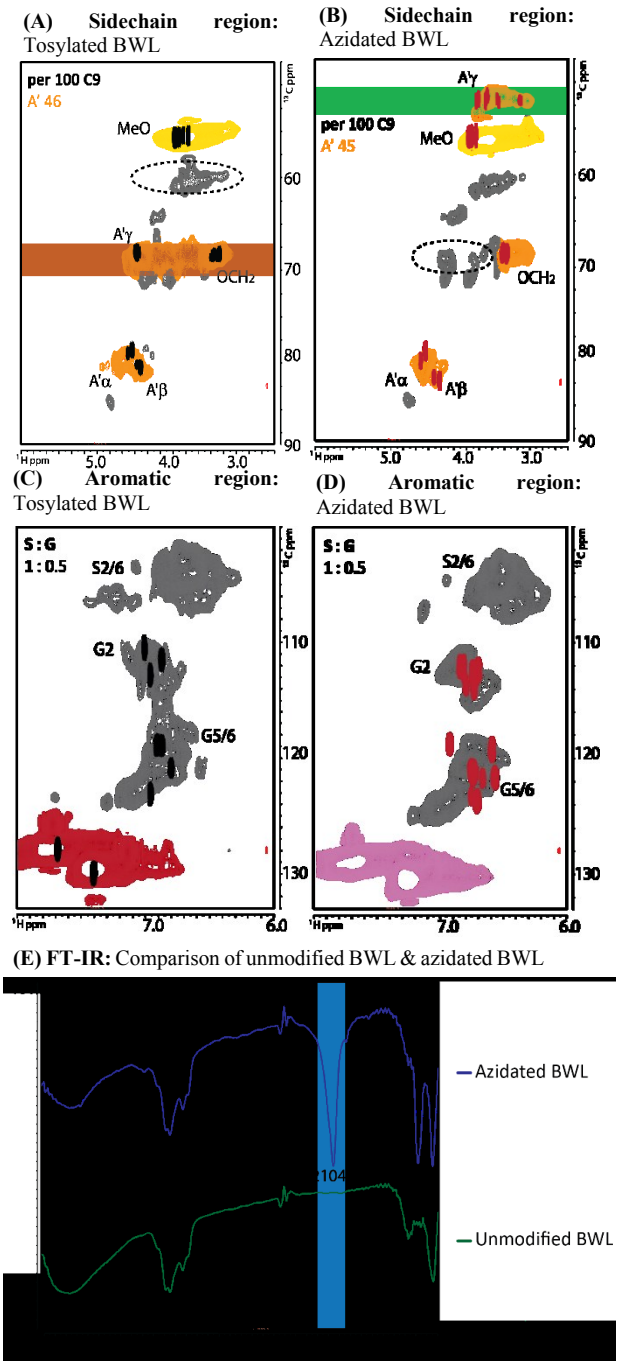


Figure 1: 2D HSQC NMR spectrum of BWL lignin overlaid with the spectra of model compounds; (**A**) linkage region of unmodified BWL overlaid with model **9** with the signals corresponding to the γ-CH_2 protons highlighted in orange (see 2D HSQC NMR analysis of the starting BWL in which the signals corresponding to the γ-CH_2 protons can be seen at (3.5-4.5, 67-71) ppm, Figure 1C); **(B)** linkage region of azidated BWL overlaid with model **13** with the signals corresponding to the γ-CH_2 protons highlighted in blue; (**C**) aromatic region of unmodified BWL with the signals corresponding to tosyl aromatics highlighted in red; **(D)** aromatic region of

azidated BWL with the signals corresponding to residual tosyl aromatics highlighted in pink; **(E)** FT-IR spectra of the starting BWL and azidated BWL with the new azide stretch highlighted in blue.

Optimization of the tosylation and azidation of BWL was required as it is often difficult to translate from model studies to lignin itself. In brief, a survey of the number of weight equivalents of *p*-toluenesulfonyl chloride identified 3.0 weight equivalents as an optimal quantity (**Figures S9** and **S10**). The azide substitution reaction was also modified from the conditions used for the models with extended reaction times being required for lignin. During this part of the study it became clear that whilst inspection of the signals corresponding to the γ-CH_2 protons indicated that complete reaction of the tosylated β-O-4 linkages had occurred (*c.f.* **Figures 1A** and **1B**, **Figure S11**), strong signals in the aromatic region that were assigned to the presence of a tosyl group, were still present (*c.f.* **Figures 1C** and **1D**). A similar observation was also reported by Yano *et al*.[23] 2D DOSY NMR analysis of azidated BWL showed that the sample was not contaminated with low molecular weight tosyl-containing compounds (**Figure S12**) and so it was proposed that the remaining tosyl signals derived from O-tosylated phenolic units in the lignin. A study using phenolic β-O-4 models was therefore carried out (**Scheme S2**). Comparison of the 2D HSQC NMR spectrum of the azidated BWL with phenolic model compounds supported the view that the remaining tosyl-derived signals observed in the azidated BWL spectrum did in fact correspond to tosylated phenolic end-groups in the lignin (**Figure S13**). As with the tosylation reaction, the azidation reaction with BWL proved reproducible (**Figure S14**).

The generality of our protocol was explored further through its application to other lignins. Two sources, Douglas fir as an example of a softwood, and an industrially relevant waste composite material provided by Fiberight Ltd. were extracted using the butanosolv pretreatment (**Figure S1D**

& **E**). **B**utanosolv **D**ouglas **F**ir **L**ignin (BDFL) and **B**utanosolv **F**iberight **L**ignin (BFL) were then successfully convert to the corresponding azides in an analogous manner to BWL (**Figures S15 - S18** and **Tables S5 – S6**). Analogous results were obtained for ethanosolv walnut shell lignin (EWL) and related model compounds (**Scheme S3** and **Figures S19** & **S20**). With a robust protocol for the production of azide-functionalized lignins in hand, we assessed the reaction with a range of click partners.

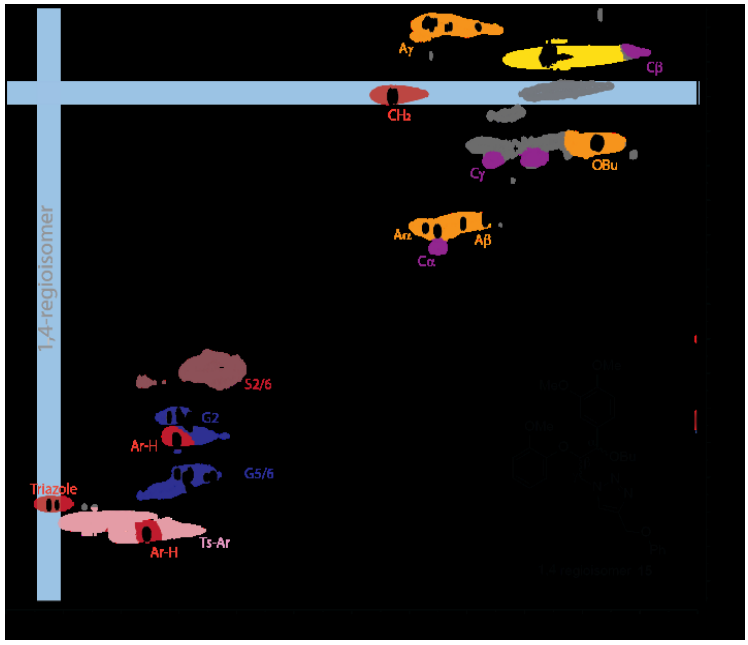
Use of CuAAC chemistry on modified lignin

In the β-O-4 model study presented above we investigated the reactivity of **5** and **13** in a copper-catalyzed azide-alkyne cycloaddition reaction and found that the triazole cycloadducts were formed in high yields, as expected. The success of the model reaction was characterized by the appearance of a distinctive signal in the 2D HSQC NMR spectra corresponding to the triazole proton (see overlaid spectrum of **15** in **Figure 2A**). When performing the CuAAC reaction on BWL, this signal was also observed (**Figure 2A**). This, along with additional changes, including a change in the chemical shift of β-O-4 γ-CH_2 signals and the presence of signals for the $PhOCH_2$ unit (*c.f.* **Figures 1A** and **1B**) supported the view that a successful reaction had occurred on lignin. Three repeats of the reaction using azidated BWL were performed to confirm its reproducibility (**Figure S21**).

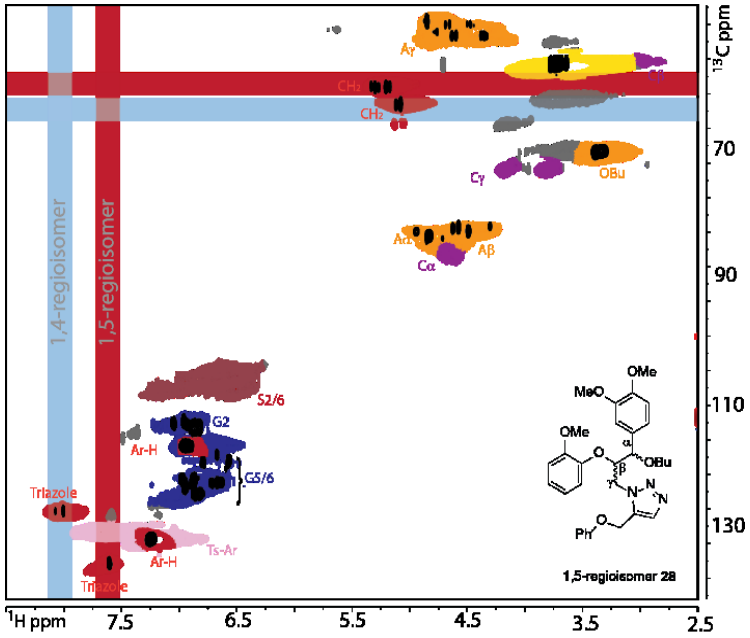
In a previous literature report, a grafted co-polymer was prepared from lignin by a *thermal* cycloaddition reaction between azidated polymer chains and a lignin that had been propargylated selectively on the phenolic positions.[13] We decided to use a *thermal* protocol with our azidated BWL and alkyne **7**. 2D HSQC NMR analysis of the resulting modified BWL confirmed formation

of both the 1,4- and 1,5-triazole isomers as expected[27] (**Figure 2B** which includes comparison to a mixture of the 1,4- and 1,5-regioisomers **15** & **20**, ESI for synthesis). It was therefore concluded that, as expected, the regioselective CuAAC variant of the reaction has advantages in producing a lignin-material which is more homogenous, with only the 1,4-triazole isomer present.

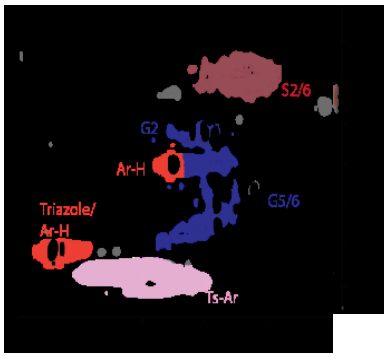
(A) 2D HSQC NMR: CuAAC BWL & model **15**



(B) 2D HSQC NMR: Thermal BWL & model **15** + **20**



(C) Aromatic Region: CuAAC BWL with *p*-NO_2 alkyne **18**



(D) Aromatic Region: CuAAC BWL with *m*-NO_2 alkyne **19**

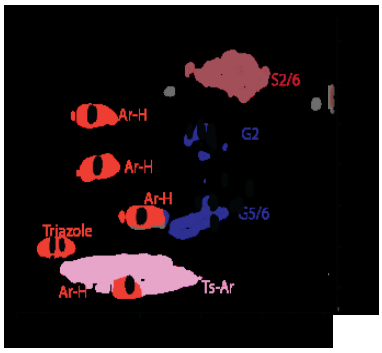


Figure 2: 2D HSQC NMR of lignin overlaid with model compounds; **(A)** BWL exposed to copper-catalyzed cycloaddition conditions using alkyne **7** (lignin general procedure **F**) overlaid with model **15**. Highlighted in blue is CH_2 of the cycloadduct and the distinctive 1,4-triazole environment;

(B) BWL exposed to thermal cycloaddition conditions using alkyne **7** (lignin general procedure **G**) overlaid with model **15** & **20** (mixture of 1,4- and 1,5-triazole regioisomers). Highlighted is the PhO$\underline{CH_2}$ of the cycloadduct and the distinctive 1,4-triazole proton environment (blue) and the additional 1,5-triazole environment (red); **(C)** BWL exposed to CuAAC conditions (lignin general procedure **F**) using 4-nitro phenyl alkyne **18** overlaid with model **16**. Highlighted in red are the overlapping aromatics from linker **18** and triazole signals; **(D)** BWL exposed to CuAAC conditions (lignin general procedure **F**) using 3-nitro phenyl alkyne **19** overlaid with model **17**. Highlighted in green are aromatics from linker **19** which have been successfully shifted away from the lignin G5/6 aromatics and the triazole environment rendering NMR analysis easier.

Additional spectroscopic evidence to support the formation of the triazole came from attempts to react azidated BWL with the nitro-containing alkynes **18** and **19** (**Scheme 1**). Whilst reaction of 4-nitro phenyl propargyl ether **18** was successful, analysis by 2D HSQC NMR proved challenging as many of the signals in the aromatic region were overlapping (**Figure 2C**), though a characteristic shift in the peak corresponding to the γ-CH_2 was still observed (**Figure S22A**). However, use of 3-nitro phenyl propargyl ether **19** led to very easy analysis as the signals corresponding to the protons in the nitro-substituted ring were shifted away from those of the lignin G5/6 aromatics (**Figures 2D** and **S22B**).

Use of the azide handles to modify the properties of butanosolv lignins

Template for co-polymer synthesis

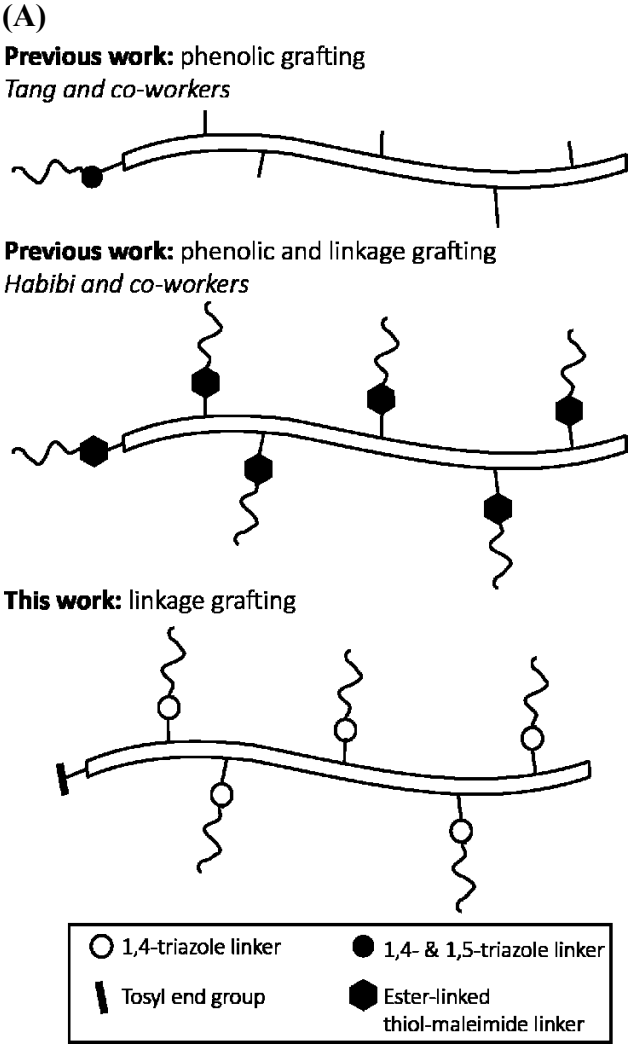
The robust and sustainable nature of the chemistry described above opens the door to a range of lignin modifications. Tang *et al.* chemically modified lignin at the phenolic positions with either alkyne or azide terminating linkers which were then coupled with complementary azide or alkyne functionalized polymers to generate block co-polymer architectures (**Figure 3A).**[13] In contrast,

Habibi et al. used thiol-maleimide click polymerization of maleimide-modified soda lignin and poly-functional thiol-containing linkers, to produce crosslinking co-polymer materials with controlled maleimide content under solvent-free, catalyst-free conditions.[28] The lignin polymeric material was characterized using 1D ^{1}H NMR and quantification using ^{31}P NMR methods. It was concluded that a brush-type architecture had been produced as the analysis showed that the phenolic and β-O-4 α- and γ-hydroxyls were all modified by labile ester formation (**Figure 3A**).[29]

In contrast, here we have used the CuAAC reaction to synthesize a novel renewable lignin-based co-polymer in which, because the BWL phenolic groups are tosylated and the β-O-4 α-hydroxyls are replaced with butyl ethers, polymer grafting can only occur selectively from the β-O-4 γ-position (**Figure 3A**). In this case we have also used monofunctional linkers, so cross-linking does not occur as it does with Habibi and coworker's lignin-copolymers.[28] In our protocol, a commercially available mono methyl etherified (poly)ethylene glycol polymer (molecular weight of around 500 Da) was modified by the incorporation of a propargyl group using literature conditions.[30] Upon exposure to CuAAC conditions with azidated BWL, a BWL-PEG brush copolymer linked via a triazole was formed as evidenced by the observation of the expected distinctive peaks in the 2D HSQC NMR analysis (**Figures S23** and **S24**). 2D DOSY NMR analysis indicated that no low molecular weight contaminants were present (**Figure S24A**) and that the PEGylated BWL material had a lower diffusion coefficient, so a higher molecular weight, than the original, azidated BWL material (**Figure S24B**). In addition, GPC analysis of the starting azidated BWL and the final PEGylated BWL showed that a significant increase in the M_w value had occurred.

Analysis of the materials produced from thermal and CuAAC-treated BWL lignins, including the PEGylated BWL material, was carried out using differential scanning calorimetry (DSC) and

thermogravimetric analysis (TGA). Melt temperatures (T_m) that were determined from the first heat on DSC measurement of the PEGylated BWL and starting BWL, showed that BWL (246 ^oC) had a lower T_m than the PEGylated BWL material (267 ^oC, **Figure 3B**).



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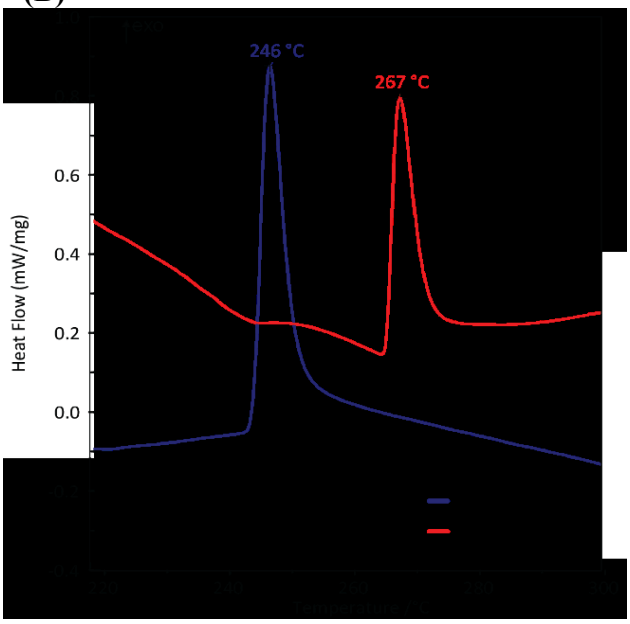


Figure 3: **(A)** Visual representation of the difference between block co-polymer structures (Tang *et al.*[13]) and brush co-polymer architectures where phenolic and linkage hydroxyls are functionalized (Habibi *et al.*[28]), or selective linkage functionalization (our work); **(B)** DSC curves from the first heat of unmodified BWL and PEGylated BWL with T_m peaks highlighted. Shown on the x-axis is temperature and the y-axis is heat flow (mW/mg) where exothermic (heat going into the sample) is shown increasing on the axis.

In addition, TGA analysis supported this observed increase in thermal stability as PEGylated BWL had a higher temperature of onset (T_{onset}) of thermal degradation (198.1 ^{o}C) compared to unmodified BWL (184.3 ^{o}C) (**Figures S25A** and **S25B**). The observed increases in T_m and T_{onset} presumably result from enhanced intermolecular interactions and polymer hydrophobicity mediated by the PEG linker incorporation. The structure of lignin itself is very heterogeneous, so it is possible that the introduction of well-defined PEG structures within our lignin-co-polymer results in regions of increased homogeneity and possible stacking leading to increased thermal stability in line with literature precedent.[31–33]

TGA analysis was also used to assess: i) the reproducibility of the CuAAC reaction between azidated BWL and alkyne **7** and ii) the materials produced either by the CuAAC or the thermal procedure. Minimal differences were observed between the three repeated copper-catalyzed cycloadditions (**Figures S25C**), suggesting our protocol is capable of reproducibly producing a material with consistent material properties. Only minor differences in the properties of the material produced by the CuAAC and the thermal reaction protocol were observed (**Figure S25D**) suggesting the incorporation of both the 1,4- and 1,5-triazole regioisomers does not significantly affect this particular material property.

Fluorescent lignin

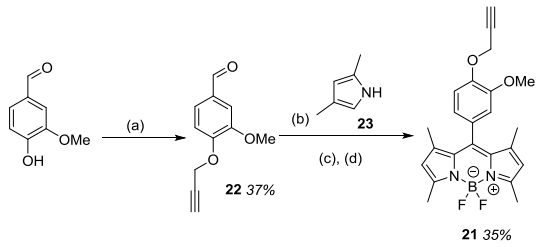
Boron-dipyrromethene (BODIPY) dyes are notable for their high quantum yield of fluorescence, which is generally independent of environmental influences.[34] For this reason these dyes are often used for imaging of biological systems[35] and labelling of polymeric systems.[36] Whilst lignin is known to be fluorescent,[37] the incorporation of a very bright and relatively photostable BODIPY-type dye significantly extends the potential utility in terms of imaging applications. We envisioned that propargylated BODIPY molecule **21** could be attached to lignin β-O-4 linkages using a CuAAC reaction. We synthesized novel BODIPY fluorophore **21** based on an existing procedure[38] with vanillin as the starting material, as it can be derived from biomass (**Figure 4A**). Fluorophore **21** was then coupled to azidated BWL and the industrially relevant composite substrate azidated BFL (**Figure S26**). Previously reported fluorescent lignins have been used in determining the distribution of lignin nanoparticles in a composite, and proposed for use as an oxygen sensor.[17]

Additionally, fluorescently-labelled monolignols introduced into the lignin biosynthetic pathway were advantageous in gaining an understanding of the lignification process.[39]

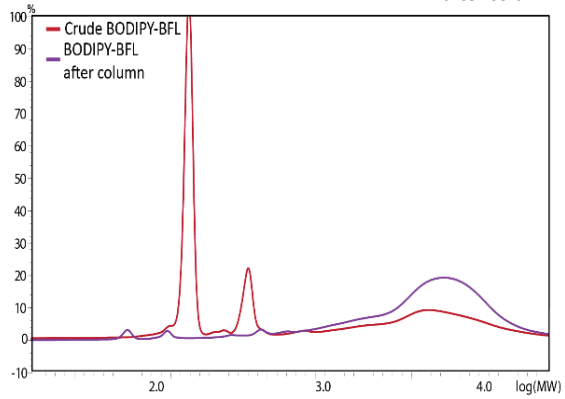
In some reactions involving lignin, we and others,[40,41] have observed that the retention of low molecular weight reactants and contaminants occurs in the lignin-derived product (**Figure 4B**). Whilst initial attempts at purifying the fluorescent lignin proved challenging (**Figure S27**), another advantage of using the butanosolv lignin was that the crude samples could be purified using standard column chromatography on silica gel. Analysis of the crude BODIPY-BFL and the purified material by GPC showed successful removal of low molecular weight impurities including **21** apparently without unintentional fractionation (**Figure 4B**). 2D DOSY NMR analysis supported that successful removal of low molecular weight contaminants had occurred (**Figure S28**).

The UV-vis absorbance spectra of **21** and BODIPY-BWL and -BFL displayed a strong absorbance in the visible part of the spectra at around 500 nm (**Figures 4C** and **S29**), similar to that previously reported for BODIPY-functionalized materials.[42,43] Fluoresence emission spectra of **21** and BODIPY-BWL and -BFL showed an emission maxima at 511 nm when excited at a wavelength of 300 nm (**Figures 4C** and **S29**). Azidated BWL and BFL showed no fluorescence emission at this wavelength.

(A) Scheme: Synthesis of vanillin derived BODIPY dye **21**



(B) GPC Traces: crude BODIPY-BFL compared to BODIPY-BFL after column



(C) UV-Vis Absorbance and Fluorescence Spectra: BFL-azide, BODIPY **29** & BFL-BODIPY

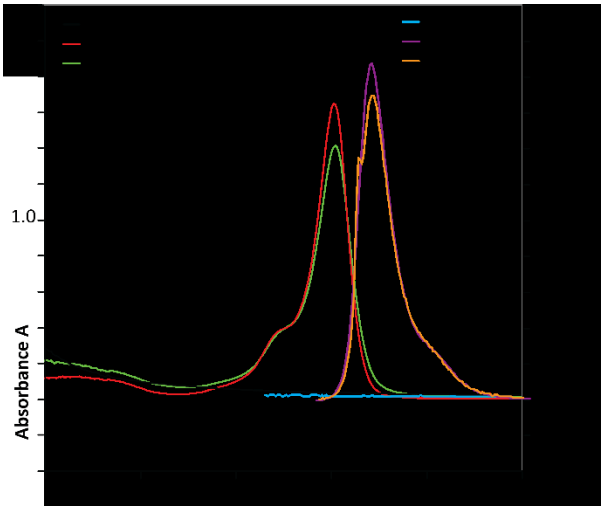


Figure 4: **(A)** Formation of novel BODIPY-derived alkyne **21** using conditions; (a) propargyl bromide, K_2CO_3, acetone, reflux, 4 hrs.; (b) pyrrole **23**, TFA, DCM, rt, 16 h; (c) chloranil, rt, 5 mins; (d) $BF_3.OEt_2$, DIPEA, rt, 2h; **(B)** GPC trace of crude BODIPY-BFL retained directly after CuAAC reaction compared with GPC trace of BODIPY-BFL after silica column chromatography; **(C)** UV-Vis absorbance spectra of azidated BFL, BODIPY dye **21** and BODIPY-BFL material after CuAAC reaction (left axis, black, red and green traces) and fluorescence emission spectra of

azidated BFL, BODIPY dye **21** and BODIPY-BFL material after CuAAC reaction (right axis, blue, purple and orange traces).

Additional analysis of the CuAAC reaction using lignin

In the final section of this study it was decided to revisit the CuAAC reaction to assess the amount of copper catalyst required. The quantitative determination of the number of β-O-4 units in a lignin sample is challenging (due to the semi-quantitative nature of standard 2D HSQC NMR protocols[44–47]), therefore the use of "weight equivalents of catalyst" is common.

We therefore decided to carry out a series of CuAAC reactions, both on BWL and BDFL with the quantity of copper catalyst used being the only variable. In brief, it was observed that loadings of as low as 0.3 weight %, which corresponds to 0.003 g of $CuSO_4.5H_2O$ per 1 g of lignin, were sufficient to allow the cycloaddition to go to completion using both BWL (**Figure S30A**) and BDFL (**Figure S30B**). Lower loadings used did not go to completion, even after extended reaction times. A consistent trend was observed, with a reduction in the signal corresponding to the azidated γ-CH_2 being tracked by the appearance of the signals previously observed (**Figures 3** and **S21**) corresponding to the γ-CH_2 and the triazole proton (**Figures S31A-O**).

Conclusions

In this work we have used an approach for the chemical modification and functionalization of organosolv lignin that involves selective tosylation of the γ-hydroxy of the β-O-4 unit followed by azide incorporation and then a CuAAC click reaction. Whilst these are established reactions in organic synthesis, they have rarely been used in the context of lignin to date and, to the best of our

knowledge, have not been used to decorate a lignin polymer along its entire length (as opposed to decoration exclusively at the phenolic end groups[13,14,48]).. The reproducibility of this protocol has been addressed at several stages. A key factor in the success of this method was the use of a butanosolv lignin and related organosolv lignins which contain high levels of α-alkoxylated β-O-4 units. Through use of relevant models, the changes in chemical environments observed by 2D HSQC NMR throughout the protocol have been rationalized. Other analytical methods, such as GPC, FT-IR, UV-Vis and DOSY-NMR have also been used to track the changes to lignin. It has been demonstrated that this protocol is applicable to the formation of sustainable lignin-based co-polymer systems with modified thermal properties, as analyzed by DSC and TGA, providing evidence for an increase in thermal stability after biopolymer modification. Our protocol was also used to link the novel BODIPY dye **21** onto the lignin backbone and to produce a novel form of fluorescent lignin. In future, we plan to explore the use of this protocol further and to assess in more detail the materials and fluorophore-related properties of the materials that can be prepared. It is hoped that it may be possible to identify a suitable application that will justify the expense that will likely be associated with the use of this protocol on an industrial scale.

ASSOCIATED CONTENT

Supporting Information. Electronic Supporting Information contains all supplementary figures mentioned throughout the text, including additional NMR spectra, GPC traces, FT-IR and UV-Vis, along with synthetic information regarding synthesis of model compounds and any novel spectra.

AUTHOR INFORMATION

Corresponding Author

Prof. Nicholas J. Westwood (njw3@st-andrews.ac.uk) Purdie Building, University of St Andrews, St Andrews, Fife, KY16 9ST.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

CuAAC, Copper-catalyzed azide-alkyne cycloaddition; p-TsCl, para-toluenesulfonyl chloride; DCM, dichloromethane; DMF, dimethyl formamide; BuOH, butanol; BWL, butanosolv walnut shell lignin; THF, tetrahydrofuran; 1^o, primary; 2^o, secondary; NMR, nuclear magnetic resonance; 2D HSQC, two-dimensional heteronuclear single quantum coherence spectroscopy; GPC, gel-permeation chromatography; FT-IR, Fourier Transform infra-red, 2D DOSY, two-dimensional diffusion-ordered spectroscopy; BDFL, butanosolv douglas fir lignin; BFL, butanosolv Fiberight lignin; EWL, ethanosolv walnut shell lignin; PEG, poly ethylene glycol; M_w, molecular weight; DSC, Differential scanning calorimetry; TGA, Thermogravimetric analysis; T_m, melt temperature; T_{onset}, temperature of onset of thermal degradation; BODIPY, boron-dipyrromethane; UV-Vis, ultraviolet-visible spectrometry; TFA, trifluoroacetic acid; wt%, weight %.

REFERENCES

(1) Zakzeski, J.; Bruijnincx, P. C. a; Jongerius, A. L.; Weckhuysen, B. M. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* **2010**, *110* (6), 3552–3599. DOI: 10.1021/cr900354u

(2) Luterbacher, J. S.; Martin Alonso, D.; Dumesic, J. A. Targeted chemical upgrading of lignocellulosic biomass to platform molecules. *Green Chem.* **2014**, *16* (12), 4816–4838. DOI:10.1039/C4GC01160K

(3) Westwood, N. J.; Panovic, I.; Lancefield, C. S. Production of Biofuels and Chemicals from Lignocellulosic Biomass, 1st ed.; Fang, Z., Richard L. Smith, J., Eds.; Springer Nature: Singapore, 2016. DOI: 10.1007/978-981-10-1965-4_7

(4) Gellerstedt, G.; Tomani, P.; Axegård, P.; Backlund, B. *Lignin Recovery and Lignin-Based Products*, RSC Green Chem.; Christopher, L., Ed.; Royal Society of Chemistry, 2013. DOI: 10.1039/9781849735063-00180

(5) Lancefield, C. S.; Rashid, G. M. M.; Bouxin, F.; Wasak, A.; Tu, W.-C.; Hallett, J. P.; Zein, S.; Rodriguez, J.; Jackson, S. D.; Westwood, N. J.; et al. An Investigation of the Chemocatalytic and Biocatalytic Valorisation of a Range of Different Lignin Preparations: the Importance of β-O-4 Content. *ACS Sustain. Chem. Eng.* **2016**, DOI: 10.1021/acssuschemeng.6b01855.

(6) Bauer, S.; Sorek, H.; Mitchell, V. D.; Ibáñez, A. B.; Wemmer, D. E. Characterization of Miscanthus giganteus lignin isolated by ethanol organosolv process under reflux condition. *J. Agric. Food Chem.* **2012**, *60*, 8203–8212. DOI: 10.1021/jf302409d

(7) Hu, G.; Cateto, C.; Pu, Y.; Samuel, R.; Ragauskas, A. J. Structural characterization of switchgrass lignin after ethanol organosolv pretreatment. *Energy and Fuels* **2012**, *26* (1), 740–745. DOI: 10.1021/ef201477p.

(8) Lancefield, C. S.; Panovic, I.; Deuss, P. J.; Barta, K.; Westwood, N. J. Pre-treatment of Lignocellulosic Feedstocks Using Biorenewable Alcohols: Towards Complete Biomass Valorisation. *Green Chem.* **2016**, 1203–1210. DOI: 10.1039/C6GC02739C.

(9) Laurichesse, S.; Avérous, L. Chemical modification of lignins: Towards biobased polymers. *Prog. Polym. Sci.* **2014**, *39* (7), 1266–1290. DOI: 10.1016/j.progpolymsci.2013.11.004.

(10) Kai, D.; Ren, W.; Tian, L.; Chee, P. L.; Liu, Y.; Ramakrishna, S.; Loh, X. J. Engineering Poly(lactide)-Lignin Nanofibers with Antioxidant Activity for Biomedical Application. *ACS Sustain. Chem. Eng.* **2016**, *4* (10), 5268–5276. DOI: 10.1021/acssuschemeng.6b00478.

(11) Kai, D.; Tan, M. J.; Chee, P. L.; Chua, Y. K.; Yap, Y. L.; Loh, X. J. Towards lignin-based functional materials in a sustainable world. *Green Chem.* **2016**, *18* (5), 1175–1200.

(12) Kai, D.; Low, Z. W.; Liow, S. S.; Abdul Karim, A.; Ye, H.; Jin, G.; Li, K.; Loh, X. J. Development of Lignin Supramolecular Hydrogels with Mechanically Responsive and Self-Healing Properties. *ACS Sustain. Chem. Eng.* **2015**, *3* (9), 2160–2169. DOI: 10.1039/C5GC02616D.

(13) Han, Y.; Yuan, L.; Li, G.; Huang, L.; Qin, T.; Chu, F. Renewable polymers from lignin via copper-free thermal click chemistry. *Polymer (Guildf).* **2016**, *83*, 92–100. DOI:

10.1016/j.polymer.2015.12.010.

(14) Xiong, K.; Jin, C.; Wu, G.; Liu, G.; Kong, Z. Preparation and characterization of lignin-silica nanoparticles via click reaction. *Chem. Ind. For. Prod.* **2016**, *36* (2), 115–120. DOI: 10.3969/j.issn.0253-2417.2016.02.017.

(15) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A stepwise huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chemie - Int. Ed.* **2002**, *41* (14), 2596–2599. DOI: 10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4

(16) Tornøe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on solid phase: [1,2,3]-Triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* **2002**, *67* (9), 3057–3064. DOI: 10.1021/jo011148j.

(17) Xiong, F.; Han, Y.; Li, G.; Qin, T.; Wang, S.; Chu, F. Synthesis and characterization of renewable woody nanoparticles fluorescently labeled by pyrene. *Ind. Crops Prod.* **2016**, *83*, 663–669. DOI: 10.1016/j.indcrop.2015.12.055.

(18) Kai, D.; Zhang, K.; Jiang, L.; Wong, H. Z.; Li, Z.; Zhang, Z.; Loh, X. J. Sustainable and antioxidant lignin-polyester copolymers and nanofibers for potential healthcare applications. *ACS Sustain. Chem. Eng.* **2017**, *5*, 6016−6025. DOI: 10.1021/acssuschemeng.7b00850.

(19) Rak, M. J.; Friščić, T.; Moores, A. H. One-step, solvent-free mechanosynthesis of silver nanoparticle-infused lignin composites for use as highly active multidrug resistant antibacterial filters. *RSC Adv.* **2016**, *6*, 58365–58370. DOI:10.1039/C6RA03711A.

(20) Jiang, S.; Kai, D.; Dou, Q. Q.; Loh, X. J. Multi-arm carriers composed of an antioxidant lignin core and poly(glycidyl methacrylate-co-poly(ethylene glycol)methacrylate) derivative arms for highly efficient gene delivery. *J. Mater. Chem. B* **2015**, *3* (34), 6897–6904. DOI: 10.1039/C5TB01202C.

(21) Ando, D.; Nakatsubo, F.; Takano, T.; Yano, H. Elucidation of LCC bonding sites via γ-TTSA lignin degradation: crude milled wood lignin (MWL) from Eucalyptus globulus for

enrichment of lignin xylan linkages and their HSQC-NMR characterization. *Holzforschung* **2015**, *70* (6). DOI: 10.1515/hf-2015-0112

(22) Diop, A.; Awada, H.; Zerrouki, R.; Daneault, C.; Montplaisir, D. Tosylation and Characterization of Lignin in Water. *Ind. Eng. Chem. Res.* **2014**, *53*, 16771−16776. DOI: 10.1021/ie502543p.

(23) Ando, D.; Nakatsubo, F.; Takano, T.; Nishimura, H.; Katahira, M.; Yano, H. Multi-step degradation method for β-O-4 linkages in lignins: γ-TTSA method. Part 3. Degradation of milled wood lignin (MWL) from Eucalyptus globulus. *Holzforschung* **2013**, *67* (8), 835–841. DOI: 10.1515/hf-2013-0126

(24) Lancefield, C. S.; Ojo, O. S.; Tran, F.; Westwood, N. J. Isolation of Functionalized Phenolic Monomers through Selective Oxidation and C-O Bond Cleavage of the β-O-4 Linkages in Lignin. *Angew. Chemie Int. Ed.* **2015**, *54*, 258–262. DOI: 10.1002/anie.201409408.

(25) Sedai, B.; Díaz-Urrutia, C.; Baker, R. T.; Wu, R.; Silks, L. A. P.; Hanson, S. K. Comparison of copper and vanadium homogeneous catalysts for aerobic oxidation of lignin models. *ACS Catal.* **2011**, *1* (7), 794–804. DOI: 10.1021/cs200149v.

(26) Ujihara, M.; Nakatsubo, F.; Katahira, R. A Novel Selective Cleavage Method for β- O -4 Substructure in Lignins Named TIZ Method. I. Degradation of Guaiacyl and Syringyl Models. *J. Wood Chem. Technol.* **2003**, *23* (March 2015), 71–87. DOI: 10.1081/WCT-120018616.

(27) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. Copper(I)-catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates. *J. Am. Chem. Soc.* **2005**, *127* (I), 210–216. DOI: 10.1021/ja0471525.

(28) Buono, P.; Duval, A.; Averous, L.; Habibi, Y. Lignin-based materials through thiol - maleimide “click” polymerization. *ChemSusChem* **2017**, *10*, 1–10. DOI: 10.1002/cssc.201601738.

(29) Liu, H.; Chung, H. Self-Healing Properties of Lignin-Containing Nanocomposite: Synthesis

of Lignin- graft -poly(5-acetylaminopentyl acrylate) via RAFT and Click Chemistry. *Macromolecules* **2016**, *49* (19), 7246–7256. DOI: 10.1021/acs.macromol.6b01028.

(30) Zhang, Q.; Ren, H.; Baker, G. L. Synthesis of a library of propargylated and PEGylated ??-hydroxy acids toward "clickable" polylactides via hydrolysis of cyanohydrin derivatives. *J. Org. Chem.* **2014**, *79* (20), 9546–9555. DOI: 10.1021/jo5016135.

(31) Back, J. F.; Oakenfull, D.; Smith, M. B. Increased Thermal Stability of Proteins in the Presence of Sugars and Polyols. *Bioch* **1979**, *18* (23), 5191–5196. DOI: 10.1021/bi00590a025.

(32) Lee, L. L. Y.; Lee, J. C. Thermal stability of proteins in the presence of poly(ethylene glycols). *Biochemistry* **1987**, *26* (24), 7813–7819. DOI: 10.1021/bi00590a025.

(33) Jiang, Y.; Ding, E.; Li, G. Study on transition characteristics of PEG/CDA solid–solid phase change materials. *Polymer (Guildf).* **2002**, *43* (1), 117–122. DOI: 10.1016/S0032-3861(01)00613-9

(34) Heisig, F.; Gollos, S.; Freudenthal, S. J.; El-Tayeb, A.; Iqbal, J.; Müller, C. E. Synthesis of BODIPY derivatives substituted with various bioconjugatable linker groups: A construction kit for fluorescent labeling of receptor ligands. *J. Fluoresc.* **2014**, *24* (1), 213–230. DOI: 10.1007/s10895-013-1289-4.

(35) Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. A Selective Turn-On Fluorescent Sensor for Imaging Copper in Living Cells. *J. Am. Chem. Soc.* **2006**, *128* (1), 10-11. DOI: 10.1021/ja055064u.

(36) Janjic, J. M.; Srinivas, M.; Kadayakkara, D. K. K.; Ahrens, E. T. Self-delivering nanoemulsions for dual in vivo fluorine- 19 MRI and fluorescence imaging. **2008**, No. 18, 2832–2841. DOI: 10.1021/ja077388j.

(37) Albinsson, B.; Li, S.; Lundquist, K.; Stomberg, R. The origin of lignin fluorescence. *J. Mol. Struct.* **1999**, *508*, 19–27. DOI: 10.1016/S0022-2860(98)00913-2.

(38) Baczko, K.; Fensterbank, H.; Berini, B.; Bordage, N.; Clavier, G.; Méallet-Renault, R.;

Larpent, C.; Allard, E. Azide-functionalized nanoparticles as quantized building block for the design of soft-soft fluorescent polystyrene core - PAMAM shell nanostructures. *J. Polym. Sci. Part A Polym. Chem.* **2016**, *54* (1), 115–126. DOI: 10.1002/pola.27772.

(39) Tobimatsu, Y.; Wagner, A.; Donaldson, L.; Mitra, P.; Niculaes, C.; Dima, O.; Kim, J. I.; Anderson, N.; Loque, D.; Boerjan, W.; et al. Visualization of plant cell wall lignification using fluorescence-tagged monolignols. *Plant J.* **2013**, *76* (3), 357–366. DOI: 10.1111/tpj.12299.

(40) Wang, Z.; Wang, X.; Fu, Y.; Li, Z.; Zhang, F.; Qin, M. Colloidal behaviors of lignin contaminants: Destabilization and elimination for oligosaccharides separation from wood hydrolysate. *Sep. Purif. Technol.* **2015**, *145*, 1–7. DOI: 10.1016/j.seppur.2015.03.001

(41) Mancera, C.; El Mansouri, N. E.; Vilaseca, F.; Ferrando, F.; Salvado, J. The effect of lignin as a natural adhesive on the physico-mechanical properties of vitis vinifera fiberboards. *BioResources* **2011**, *6* (3), 2851–2860.

(42) Carpenter, B. L.; Scholle, F.; Sadeghifar, H.; Francis, A. J.; Boltersdorf, J.; Weare, W. W.; Argyropoulos, D. S.; Maggard, P. A.; Ghiladi, R. A. Synthesis, Characterization, and Antimicrobial Efficacy of Photomicrobicidal Cellulose Paper. *Biomacromolecules* **2015**, *16* (8), 2482–2492.

(43) Goze, C.; Ulrich, G.; Mallon, L. J.; Allen, B. D.; Harriman, A.; Ziessel, R. Synthesis and photophysical properties of borondipyrromethene dyes bearing aryl substituents at the boron center. *J. Am. Chem. Soc.* **2006**, *128* (31), 10231–10239.

(44) Heikkinen, S.; Toikka, M. M.; Karhunen, P. T.; Kilpeläinen, I. A. Quantitative 2D HSQC (Q-HSQC) via suppression of J-dependence of polarization transfer in NMR spectroscopy: Application to wood lignin. *J. Am. Chem. Soc.* **2003**, *125* (14), 4362–4367.

(45) Koskela, H.; Kilpeläinen, I.; Heikkinen, S. Some aspects of quantitative 2D NMR. *J. Magn. Reson.* **2005**, *174* (2), 237–244.

(46) Koskela, H.; Heikkilä, O.; Kilpeläinen, I.; Heikkinen, S. Quantitative two-dimensional HSQC experiment for high magnetic field NMR spectrometers. *J. Magn. Reson.* **2010**, *202*

(1), 24–33.

(47) Peterson, D. J.; Loening, N. M. QQ-HSQC: A quick, quantitative heteronuclear correlation experiment for NMR spectroscopy. *Magn. Reson. Chem.* **2007**, *45* (11), 937–941.

(48) Jin, C.; Zhang, X.; Xin, J.; Liu, G.; Wu, G.; Kong, Z.; Zhang, J. Clickable synthesis of 1,2,4-triazole modified lignin-based adsorbent for the selective removal of Cd(II). *ACS Sustain. Chem. Eng.* **2017**, *5* (5), 4086-4093.

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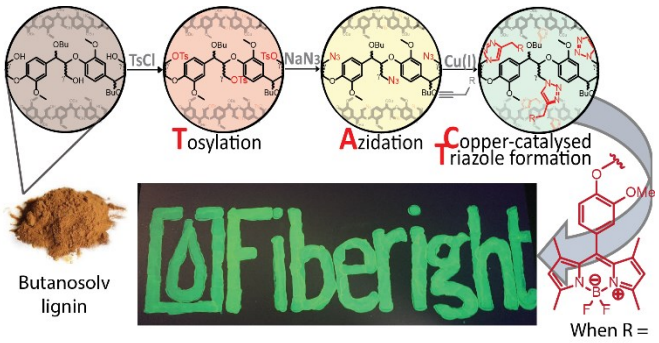

SYNOPSIS

A tosylation, azidation, copper-catalyzed triazole formation protocol has been developed to allow grafting of copolymers and molecules onto technical lignins.